SENSITIVITY OF *N. GONORRHOEAE* TO ANTIBIOTICS*

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Since the late thirties, physicians have been in a very fortunate position with regard to the therapy of gonorrhoea. At that time the sulphonamide drugs were discovered and produced a revolution in the treatment of gonorrhoea; when the incidence of resistant gonococcus strains became too high, penicillin came into the picture, closely followed by streptomycin and tetracycline. In addition, other antibiotics have a considerable effect on the gonococcus *in vivo* and *in vitro* (chloromycetin, erythromycin, oleandomycin, syntomycin, novobiocin, kanamycin, etc.); yet, in spite of the decreasing clinical effect and the increasing incidence of allergic response, the drug of choice has been and still is penicillin. Certain disadvantages exist for most of the other antibiotics, and they cannot be recommended, at least not for large-scale routine treatment or for use alone. Factors relevant in this connexion are: cost, method of application, toxicity, tendency to development of less sensitive or resistant strains, and selective effect on other bacteria such as the staphylococci and klebsiellae.

It is well known that complete resistance to streptomycin develops very easily, and it was recently shown by Meyer-Rohn (1958) that gonococci can be made completely resistant to erythromycin and oleandomycin, and that some resistance to tetracycline can also be developed.

In this paper the problems of sensitivity and treatment are considered mainly from the bacteriological point of view. The considerations are based upon work done at the Statens Seruminstitut in Copenhagen and it is not intended (to any great extent) to refer to other similar work. The gonococcus department of the Statens Seruminstitut in Copenhagen serves as a centre for the entire country, about 100,000 specimens being received each year; about 10–12 per cent. result in positive cultures, 80

per cent. being from women and 20 per cent. from men (10,000 to 12,000 positive cultures per year).

Penicillin

Late in 1955 four gonococcal strains were isolated from a case of uncomplicated gonorrhoea which was refractory to repeated "ordinary" penicillin treatment; all four strains showed a sensitivity to penicillin twenty times lower than usual. The lowered sensitivity persisted, and after the usual dose of penicillin the patient's serum showed levels similar to those commonly observed. This observation, together with the increasing use of oral penicillin, and the increasing number of allergic cases, made it desirable to review the sensitivity of gonococci to penicillin and to other possible antibiotics. In addition, a few reports were found from other countries, in which a change in the distribution of the *in vitro* concentrations necessary for complete *in vitro* inhibition was described.

Preliminary testing by means of the tablet method of 300 strains isolated in 1956 showed that 10 per cent. of these strains were about twenty times less sensitive than usual.

The department possessed a collection of freeze-dried strains, among which a high number had been isolated and dried before the penicillin era, and it was decided to compare the sensitivity of strains isolated until 1944 with that of strains isolated in 1957. The sensitivity to streptomycin and tetracycline was also determined.

The relation between the *in vitro* and the *in vivo* results was investigated by discriminating between strains sent in for diagnostic purposes only and strains with a request for drug-sensitivity determination. Two methods were employed: a tablet or diffusion method, and a plate dilution method; they agreed well, but the latter gave more accurate results. Therefore, most of the results which are

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presented below are from the plate dilution method (Reyn, Korner, and Bentzon, 1958).

The medium used was McLeod's "chocolate" agar enriched with ascitic fluid; the antibiotics were added to the medium in two-fold dilutions. The inoculum was about 10^6 organisms spread on a square centimetre or so. The results were read after 48 hours' incubation in a CO_2 atmosphere; the growth on the control plates without antibiotic was called four plus and the decreasing degrees of growth on the other plates were read as three plus, two plus, and one plus. Four plus was counted as 100 per cent., three plus as 75 per cent., and so on. The 50 per cent. inhibitory concentrations were calculated by means of the so-called Kärber method as $\log_{10}$ to the base ten values (Finney, 1947).

The dose response curves were rather steep; generally complete inhibition needed half as much again as was needed for 50 per cent. inhibition. It was found that the 50 per cent. value could be estimated more precisely than the 100 per cent. value.

In the tablet method, the plates were inoculated by flooding the surface with a saline suspension of about 10^8 organisms per ml. Tablets with a known content of antibiotic were put on and the plates were incubated for 24 hours. The diameters of the inhibition zones were measured in mm, and the results were recorded in mm. The penicillin tablets contained 25 units or 15 μg . penicillin G.

In Fig. 1 the distribution of the ninety 1944 strains in relation to the 50 per cent. inhibitory concentrations of penicillin is given; the distribution is "normal" with a peak corresponding to $\cdot 01 \mu\text{g}$. with the range from $\cdot 004$ to $\cdot 024 \mu\text{g}$.

Fig. 2 (opposite) gives the distribution of the 103

1957 strains sent in for diagnosis ("diagnostic" strains). Here we have two peaks: one at $\cdot 01 \mu\text{g}$. as in Fig. 1 and one at about $\cdot 2 \mu\text{g}$. with the range from $\cdot 006$ to $\cdot 4 \mu\text{g}$. Below, the distribution is shown of the 1957 strains which were accompanied by a request for their sensitivity ("sensitivity" strains). Here also, there are two peaks, but the peak at about $\cdot 2 \mu\text{g}$. is larger than that in the top diagram. The range is from $\cdot 003$ to $\cdot 5$. The strains fall into two groups with penicillin.

The two-peak distributions, which can also be derived from the figures in several recent British papers, suggest a stepwise increase in resistance (Cradock-Watson, Shooter, and Nicol, 1958; King, 1958; Curtis and Wilkinson, 1958).

Among the strains sent in for diagnosis, the incidence of 50 per cent. inhibitory value greater than $\cdot 036 \mu\text{g}$. is 17 per cent. compared with 46 per cent. among the strains sent in for sensitivity testing. This difference is significant and shows a correlation between clinical failure of treatment and "resistance" *in vitro*. 30 per cent. of the 1957 strains needed more than $\cdot 075 \mu\text{g}$. for complete inhibition and 18 per cent. needed $\cdot 3 \mu\text{g}$. or more. None of the 1944 strains needed more than $0\cdot 035 \mu\text{g}$.

Several recent reports show a correlation between the *in vivo* and *in vitro* findings; the reports are mainly from the United States of America, Great Britain, the Netherlands, and Scandinavia.

Since 1957 the sensitivity level has been followed for penicillin, using the tablet method in the routine testing of strains for which a drug-sensitivity determination was requested, and altogether 583 "sensitivity" strains were tested.

Through the years the range of zone diameters was unchanged; it went from 20 to 52 mm. Strains

FIGURE 1

FIGURE 2

with zones less than 34 mm. were designated as less sensitive. In the years from 1957 to 1960, the proportion of strains with reduced sensitivity was practically unchanged or only slightly increasing; it went from 39 per cent. in 1957 to 43.5 per cent. in 1960.

In February, 1960, the dilution method was used again; Fig. 3 (overleaf) shows the sensitivity distribution of 265 "sensitivity" strains; there is still a two-peak distribution with the same range as that found in 1957 (≤ 0.0039 to 0.50 µg./ml.; the "broken columns" indicate values ≤ 0.0039 µg./ml.).

The incidence of strains with 50 per cent. inhibitory value greater than 0.036 µg./ml. is 51 per cent. against 46 per cent. in 1957. The sensitivity distribution was also determined for 102 "diagnostic" strains received in September and October, 1960; they ranged from 0.0039 to 0.84 µg./ml. Only 20 per cent. showed 50 per cent. inhibitory values greater than 0.036 µg./ml. Thus, the difference demonstrated in 1957 between "diagnostic" and "sensitivity" strains was found again in 1960 (Fig. 4, overleaf).

These results agree with those obtained with the

tablet method and indicate—perhaps—that it will take a relatively long time before all strains belong to the less sensitive group. Yet it is noteworthy that the percentage of less sensitive strains has increased for both "diagnostic" and "sensitivity" strains.

In addition to the selective and perhaps mutagenic effect of penicillin on the sensitivity, other possible effects have been observed. Antibiotic treatment may lead to selection not only directly by favouring the more resistant clones, but also *indirectly by killing the most easily available organisms*. Organisms protected either by phagocytosis or by inflammatory tissue may be spared. Suggestive factors are the prolonged incubation period in males reported by several authors and the less pronounced symptoms when compared with classical gonorrhoea. The protection against penicillin of gonococci phagocytosed in tissue culture also supports the above-mentioned possibility (Thayer, Field, Magnuson, and Garson, 1957; Thayer, Perry, Magnuson, and Garson, 1957; Thayer, Perry, Field, and Garson, 1957).

FIGURE 3

In June, 1957, a striking change in the type of growth was observed; a high proportion of the strains grew very poorly and either did not ferment glucose or showed only a weak fermentation (Reyn and Bentzon, 1959, 1961). These atypical strains could not be classified as *N. catarrhalis*, the only non-pigmented species in the *Neisseria* group which does not ferment glucose; they did not grow at 22° C. The colonial morphology and other characteristics including serology were like those of gonococci; they were oxidase positive. In complement-fixation experiments it was found that nearly all the strains yielded anticomplementary antigens; however, this effect was counteracted by heated serum, which indicated that the anti-complementary effect may be ascribed to inactivation of either the third or fourth component of complement or of both. Altogether fifty atypical strains were tested sero-

logically and they all gave positive reaction with pooled, human antigenococcus serum. Before June, 1957, only about 1–2 per cent. of the satisfactorily growing strains isolated in the routine were unable to ferment glucose and, only rarely, non-fermenting strains were isolated with a very poor growth on the routine medium as well as the fermentation medium.

The occurrence of "atypical" strains with "delicate" growth has previously been described by Morton and Shoemaker (1945) and Reyn (1948); Reyn found then that the majority of the atypical strains showed typical fermentation on repeated subculture. The normal fermentation occurred often in the way that normally fermenting, "coarse" colonies appeared among the "delicate", non-fermenting colonies, indicating a mutation.

The atypical non-fermenting strains now observed grew very poorly on the routine media for isolation

FIGURE 4

and fermentation. After 48 hours' growth the colony-diameters were about 0.25 to 0.5 mm. only. By viability counting of diluted, standardized cultures it was found that the *viability of the atypical strains was from five to twenty times less than that of the normal strains.*

At first it was hoped that repeated subculture would result in "back mutation" so that fermentation would be normal, but this was not the case. It became necessary to solve the problem otherwise by improving the media and by supporting the bacteriological diagnosis by means of serological testing.

Fig. 5 shows the weekly percentage distribution of patients with (A) non-fermenting strains and (B) slightly + non-fermenting strains. Only the non-fermenting strains were registered from the onset of the "troubles", but from 7 July, 1957, both the slightly- and the non-fermenting strains were registered. No constant difference is found between the two curves; however, when the incidence of non-fermenting strains is high there is also a high incidence of slightly-fermenting strains and *vice versa*.

The peak of non-fermenting strains in June,

FIGURE 5

1957, is somewhat artificial; to obtain typical fermentation in new specimens two or three subsequent cultures were often received per patient. Later on, when the practitioners knew that the non-fermenting strains were to be considered as "true" gonococci, Fig. 5 reflects the actual incidence of the atypical strains. The figure shows that three small epidemics occurred during the summer and autumn of 1957; in January, 1958, the incidence rose considerably (to about 40 per cent.) and this level was maintained until May, 1958, when we finally succeeded in improving our media. The atypical strains were found repeatedly in the same patients and in their contacts. No efforts were made to follow the cases epidemiologically; however, cases appeared all over the country at once.

Several unsuccessful attempts were made to improve the media; in April, 1958, it was found by mere accident that the use of ox-heart broth instead of ox-meat broth resulted in larger colonies and somewhat more distinct fermentation. However, ideal fermentation was not obtained until human placenta broth was used in the fermentation plates. So far, we have no explanation for the better results. Old typical strains (isolated in 1944) and new strains (typical and atypical) grow equally well on the improved media, *viz.* prepared with ox-heart broth for primary isolation and with human placenta broth for fermentation. The viability of standardized cultures of atypical strains was not improved on the new medium.

Two years have now passed since the media were changed and the atypical growth was got rid of; since then only non-fermenting strains from 38 patients (or from about 0.4 per cent. of all the patients) have been found.

The atypical strains were *preponderantly sensitive to both penicillin and streptomycin* as measured with the tablet method. They tended to be more sensitive to penicillin than did the typical strains. It is suggested that the appearance of the atypical strains is a result of penicillin therapy, in spite of their tendency to increased penicillin sensitivity as compared to other strains isolated in the same year. The atypical strains may have been evoked by penicillin because the action of penicillin is closely connected with the division process (*i.e.* Lederberg, 1950, 1957).

Stability of the in vitro sensitivity to penicillin

Twenty strains with reduced sensitivity to penicillin were subcultured daily for 30 to 90 days on "chocolate" medium without penicillin; about 10⁷ viable organisms were spread daily on fresh plates. The sensitivity of these subcultured strains was

compared with that of the same strains maintained in lyophilized cultures and reconstituted in the same medium 1 or 2 days before the experiment. The strains were tested to penicillin, streptomycin, and tetracycline using the tablet method, and to penicillin using the dilution method.

Tablet Method.—A tendency to increased zone-diameters for the strains which had been subcultured was found; the diameters of the subcultured strains were on the average about 2 mm greater than those of the lyophilized strains. However, this applied to all three antibiotics and the differences between the zones were associated with the strain in particular, indicating that changed growth requirements might be the cause and not differences in sensitivity. The range of the differences in the diameters was found —3.7 to +7.0 mm.; seventeen of the differences were positive (9 significantly) and three were negative (2 significantly).

Dilution Method.—No significant differences were observed between the penicillin sensitivity of the two sets of strains. It was also found—in spite of frequent subculture—that the control strain with reduced penicillin sensitivity had kept its penicillin sensitivity level for more than 2 years.

Streptomycin

Of the ninety strains isolated in 1944, 88 were tested with streptomycin; the distribution of 50 per cent. inhibitory concentrations was normal with a range of 3 to 11 µg. per ml.

Fig. 6 (opposite) shows the distribution with streptomycin for the 1957 strains sent in for diagnosis; there are two peaks, one at 3 µg. and one at 6 µg.; one strain was very resistant, and withstood more than 2,000 µg.

Fig. 7 (opposite) shows the distribution of strains sent in for sensitivity; here also there are two peaks, but the peak at 6 µg. is higher than that in Fig. 6.

Three very resistant strains were found; thus, with streptomycin, 38 per cent. of the "diagnostic" strains and 64 per cent. of the "sensitivity" strains had a 50 per cent. inhibitory value greater than 3.7 µg. This is a significant difference. The four very resistant strains all came from patients previously treated with streptomycin.

As with penicillin, the sensitivity level was followed by means of the tablet method. The tablets contained 3 mg. streptomycin.

FIGURE 6

FIGURE 7

The incidence of "sensitivity" strains resistant to streptomycin has been distinctly increasing; in January–February, 1960, 11 per cent. were completely resistant. In addition it was found that the range of diameters showed a tendency towards smaller zones for 1959 and 1960 as compared with 1957 and 1958.

In February the dilution method was used again and Fig. 3 (p. 148) shows that 26 per cent. of the strains sent

in with a request for sensitivity determination were resistant to more than 2,000 µg./ml. The range of the other strains was about the same as that found in 1957, namely from less than 0.71 to 8 µg./ml. 10 per cent. of 102 "diagnostic" strains from 1960 were resistant to more than 2,000 µg./ml. The other strains ranged from 0.84 to 4.75 µg./ml. (The "broken columns" indicate values ≤ 0.71 and ≤ 1.41 µg./ml.).

For streptomycin it is a question of either sensitivity or complete resistance. This is in accordance with what has been found for other bacteria. Streptomycin is not very much used in Denmark and the increased number of resistant strains must either be the result of a few physicians' experiments with this drug or the result of imported strains from other countries, especially France and South America. Roiron, Rasetti-Nicod, and Durel (1961) have recently found that 25 per cent. of French gonococci are resistant to streptomycin. Alergant (1958) has also found increasing incidence of resistant strains (2.3 per cent. in 1954 and 7 per cent. in 1956) in Liverpool.

Tetracycline

The strains from 1944 showed a sensitivity range from 0.4 to 0.9 $\mu\text{g./ml.}$ and, irrespective of their origin, the strains from 1957 showed a one-peak distribution at about 0.6 $\mu\text{g./ml.}$ The value ranged from 0.1 to 1 $\mu\text{g./ml.}$ (10-fold). In 1950 the sensitivity range of 208 strains was from ≤ 0.12 to 3.0 $\mu\text{g./ml.}$ (≤ 11 -fold), with most values around 0.3 $\mu\text{g./ml.}$ 102 "diagnostic" strains showed a similar distribution with a range from 0.135 to 1.5 $\mu\text{g./ml.}$ Not very

much seems to have happened on this front and this is in accordance with the fact that the tetracyclines are not very much used (Figs 8 and 9). However, quite recently an increasing number of strains which require more than 2 $\mu\text{g./ml.}$ for 100 per cent. inhibition have been observed (Fig. 9), viz. 10 per cent. of 351 "sensitivity" strains received from September to December, 1960, against only 4 per cent. of the 208 "sensitivity" strains received from February to August, 1960.

Sulpha Drugs

Only a few years after the sulpha drugs were introduced into therapy they proved to be practically useless. They have not been used to any large extent for about 15 years and the sensitivity distribution of *N. gonorrhoeae* appears to be the same as that found before sulpha therapy had been used.

In Fig. 10 and in the Table (overleaf) a number of results of sensitivity testings of strains isolated since 1925 is presented. Most of the figures are from Denmark and other Scandinavian countries, and there are also some from the United States of America and England, but the list is not claimed to be complete.

FIGURE 9

The black dots in Fig. 10 correspond to determinations *in vitro* by means of the dilution method, and the white triangles for determination *in vivo*. The results *in vitro* and *in vivo* are all shown as black triangles. It is evident that it took only 5 to 6 years to make nearly all the strains resistant; and it took perhaps a little longer to get back to the pre-treatment level. The results are only directly comparable for the Danish figures and it is emphasized that, in addition to the 3 per cent. completely resistant strains, 31 per cent. of the 1960 strains showed decreased sensitivity, indicating that altogether 34 per cent. of the strains would not respond to ordinary sulpha treatment, although they might possibly respond to treatment with slowly-excreted sulpha drugs.

Fig. 8 (p. 152) shows the 1960 distribution of the 50 per cent. inhibitory concentration for "sensitivity" strains; the range is wide, namely from 0.6 to 144 $\mu\text{g./ml.}$ or 190-fold; 3 per cent. of the strains were resistant needing more than 96 $\mu\text{g./ml.}$ for complete inhibition. The distribution is fairly even over this range with a flat top at about 3 $\mu\text{g./ml.}$ It is well known that the variance of sulpha drug determinations is much higher than that for penicillin and it is

possible that this fact would tend to blur the picture.

1 per cent. of the 102 "diagnostic" strains from 1960 showed a 50 per cent. inhibitory concentration higher than 48 $\mu\text{g./ml.}$ and 34 per cent. needed more than 6 $\mu\text{g./ml.}$; they ranged from 0.32 to 57 $\mu\text{g./ml.}$ (Fig. 9).

Correlations

In a previous paper (Reyn, Korner, and Bentzon, 1958), a positive correlation was found between the degrees of sensitivity to penicillin and streptomycin of strains isolated in 1957. Also the streptomycin and tetracycline values, and the tetracycline and penicillin values were positively correlated.

The 1960 values for the "sensitivity" strains were statistically* treated and significant positive correlations were again found between penicillin, streptomycin, and tetracycline. It was also found that the 50 per cent. inhibitory concentrations of the three drugs were positively correlated with the values for sulphathiazole, which had not been included in the previous investigation. It is noteworthy that, of a total of 265 "sensitivity" strains, 68 required more than 2,000 $\mu\text{g. streptomycin per ml.,}$ and that all the

* I wish to thank Mr. S. Olesen Larsen for valuable statistical help.

FIG. 10.—Sensitivity distribution to sulphathiazole of gonococcal strains isolated from 1925–35 to 1960. The numbers correspond to the numbers given in the Table. No distinction has been made between “sensitivity” and “diagnostic” strains, with the exception of the results from the present paper.

TABLE
SENSITIVITY OF *N. GONORRHOEAE* TO SULPHATHIAZOLE, 1925–1960

Authors	Date	No.	Country	Date Strains Isolated	No. of Strains or Patients	Method		Percentage Resistant	Complete Inhibition (µg./ml.)	Drug
						<i>in vivo</i>	<i>in vitro</i>			
Schmith and Reymann	1940	1	Denmark	1925–36	50	No	Dilution	8	50	Sulphapyridine
Nørgaard	1946	2	Denmark	1939–40	479	Yes	Not done	22		Sulphathiazole
Schmith and Reymann	1940	3	Denmark	1940	355	Yes	Dilution	20	50	Sulphapyridine
Hagerman	1942	4	Sweden	1941–42	175	Yes	Dilution	21	64	Sulphapyridine
Marcussen	1944	5	Denmark	1944	511	Yes	Dilution	13–40 (27)	50	Sulphathiazole
Selberg	1945	6	Norway	1945	126	Yes	Not done	54		Sulphathiazole
Nørgaard	1946	7	Denmark	1944–46	1,487	Yes	Not done	59		Sulphathiazole
Lindau	1945	8	Sweden	1944–45	89	Yes	Dilution	76	64	Sulphathiazole
Dunlop	1949	9	England	1946–47	205	Yes	Not done	86		Sulphathiazole
Gocke and Others	1950	10	U.S.A.	1948	63	No	Dilution	84	100	Sulphadiazine
Del Love and Finland	1955	11	U.S.A.	1954	43	No	Dilution	11	100	Sulphadiazine
Cradock-Watson and Others	1958	12	England	1957	200	No	Dilution	1	8	Sulphathiazole
Thayer and Others	1957	13	U.S.A.	1957	43	No	Dilution	3	100	Kynex and Sulphadiazine
Reyn—This paper	1961	14a	Denmark	1960	265*	Partly	Dilution	3	96	Sulphathiazole
		14b	Denmark	1960	102†	Partly	Dilution	1	96	Sulphathiazole

* Sensitivity. † Diagnosis.

strains resistant to streptomycin needed more than 0.15 µg. (0.25 I.U.) penicillin for complete inhibition.

It is natural to ascribe the positive correlation between all four antibiotics to a successive selection in the same way as the extended use of penicillin has led to a selection of strains relatively resistant to this

drug. Patients infected by such strains have then been treated with streptomycin (or *vice versa*) and in case of relapse with tetracycline or sulphathiazole. It is possible that a sensitivity change may occur with two or more of the antibiotics at the same time, but the *in vitro* experiments hitherto presented are against this assumption.

Concluding Remarks

In Denmark the antibiotic situation may be characterized as follows:

The sensitivity level for *penicillin* is nearly the same to-day as in 1957. About 30 per cent. of all the gonococcus strains need ≤ 0.075 $\mu\text{g.}$ penicillin per ml. for complete *in vitro* inhibition; the highest concentration needed is about 1.5 $\mu\text{g./ml.}$ (2.5 I.U.), but this has been found only for a few strains isolated in August, 1960. Thus, it should be possible to cure all cases of gonorrhoea by means of some kind of penicillin treatment, except when the patient is allergic to this drug.

To-day *streptomycin* would fail to cure about 15 per cent. of all cases and, if it was used to any large extent, all gonococcus strains would rapidly become absolutely resistant.

Sulphathiazole would fail to cure at least 35 per cent. and perhaps more.

It is difficult to predict what would happen if *tetracycline* were used as the only treatment; we have recently gained some evidence which indicates that less sensitive strains are appearing.

The therapeutic details such as choice of drug or method of administration depend, among other things, on the average concentration obtainable in the tissues and the length of time for which this concentration is maintained. Clinical experience must also be considered and the treatment schedule or schedules must be determined by collaboration between bacteriologists, pharmacologists, and physicians. To counteract the selective effect of antibiotic treatment the use of combined therapy might be considered.

The findings *in vitro* depend on the method and especially on the kind of medium used; it would be very valuable if a *reference procedure* involving the use of reference strains could be agreed upon. This applies to sensitivity determinations of all micro-organisms, and the problem has recently been discussed by the W.H.O. Expert Committee on Antibiotics, Geneva, July 11–16, 1960.

Summary

950 gonococcal strains, isolated in 1958, 1959 and 1960, have been tested for their sensitivity to penicillin, streptomycin, tetracycline, and sulphathiazole. A tablet method was used until February, 1960, and after that time a plate dilution method was used. The results of the tablet method were read in millimetres and those of the plate dilution method were determined as the 50 per cent. inhibitory concentration expressed in $\mu\text{g./ml.}$

Penicillin.—583 strains accompanied by a request for drug sensitivity ("sensitivity" strains) were isolated in the period from January, 1958, to February, 1960; they were tested with the tablet method. The sensitivity distribution was similar to that found for strains isolated in 1957, although with a tendency towards a higher proportion of less sensitive strains. 265 "sensitivity" strains isolated from February to August, 1960, were tested with the plate dilution method. The 50 per cent. inhibition concentrations showed a two-peak distribution with one peak at about 0.01 $\mu\text{g./ml.}$ and the other at about 0.2 $\mu\text{g./ml.}$ They varied over about the same range as that previously found for strains isolated in 1957; 51 per cent. showed 50 per cent. inhibitory concentrations greater than 0.036 $\mu\text{g./ml.}$ against 46 per cent. of this category for the 1957 strains. The corresponding values for 102 strains sent in for diagnosis ("diagnostic" strains) were 20 and 17 per cent. respectively.

Thus, the previously observed statistically significant difference between "sensitivity" and "diagnostic" strains was confirmed; in addition, a tendency towards a higher proportion of less sensitive strains was observed (Figs 3 and 4).

Streptomycin.—The same strains as those tested to penicillin were tested to streptomycin. With the tablet method a sudden increase to 11 per cent. of resistant strains was noted in January and February, 1960; with the plate dilution method 26 per cent. of the 265 "sensitivity" strains were found to be resistant, whereas only 10 per cent. of the 102 "diagnostic" strains were resistant (Figs 3 and 4). For complete inhibition the resistant strains required more than 2,000 $\mu\text{g./ml.}$

Tetracycline.—208 "sensitivity" and 102 "diagnostic" strains were tested with the plate dilution method. Both groups fell within the same range as that previously observed for strains isolated in 1957. More recently, however, a tendency to increasing 50 per cent. inhibition concentration was observed for 351 "sensitivity" strains received from October to December, 1960. 4 per cent. of the 208 "sensitivity" strains isolated first required ≥ 1.05 $\mu\text{g./ml.}$ for 50 per cent. inhibition, whereas 10 per cent. of 351 more recently isolated strains had the same requirement (Figs 8 and 9).

Sulphathiazole.—Previous investigations (mainly Scandinavian) were reviewed and presented in the Table and Fig. 10 together with the author's latest results. In 1954 the sensitivity level was found to be about the same as that observed before the sulphonamides were introduced into therapy.

When tested with the plate dilution method, the 265 "sensitivity" strains varied over a nearly 200-fold range. 3 per cent. were resistant requiring ≤ 48 $\mu\text{g./ml.}$ for 50 per cent. inhibition; 31 per cent. were "less sensitive" requiring between 8.5 and 48 $\mu\text{g./ml.}$ for 50 per cent. inhibition. The corresponding values for the 102 "diagnostic" strains were 1 and 38 per cent. respectively (Figs 8 and 9).

Correlations.—Positive and statistically significant correlation was found between the inhibitory concentrations of all four antibiotics. Further, it is worthy of mention that 68 "sensitivity" strains required more than 2,000 $\mu\text{g. streptomycin per ml.}$ for complete inhibition; all these strains required at least 0.15 $\mu\text{g. penicillin per ml.}$ for complete inhibition.

With a view to the therapeutic consequences, the significance of the observed increase in the frequencies of less sensitive and resistant strains is discussed. It is underlined that the physician, the pharmacologist and the bacteriologist should collaborate in order to utilize in the best possible way the advantages offered by the modern antibiotics.

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Sensibilité de *N. gonorrhoeae* aux antibiotiques

Résumé

L'auteur a étudié la sensibilité à la pénicilline, à la streptomycine, à la tétracycline et au sulfathiazole de 950 souches gonococciques isolées en 1958, 1959 et 1960. Elle s'est servi de la méthode de la tablette jusqu'en février 1960, et après cette date, elle employa celle de la dilution sur plaque. Les résultats de la méthode de la tablette furent exprimés en millimètres et ceux de la méthode de la dilution sur plaque sous forme de concentration inhibitrice à 50 % exprimée en $\mu\text{g./ml.}$

Pénicilline: L'auteur reçut 583 souches, accompagnées d'une demande de détermination de sensibilité à la drogue (souches "à déterminer"), isolées de janvier 1958 à février 1960; elles furent examinées par la méthode de la tablette. La distribution de la sensibilité était similaire à celle des souches isolées en 1957 avec, cependant, une proportion plus élevée de souches moins sensibles. 265 souches "à déterminer", isolées de février 1960 à août 1960, furent examinées par la méthode de la dilution sur plaque. La courbe des concentrations inhibitrices à 50 % accusait deux sommets, l'un à environ 0,01 $\mu\text{g./ml.}$ et l'autre à environ 0,2 $\mu\text{g./ml.}$ Les variations étaient à peu près les mêmes que pour les souches isolées en 1957; 51 % avaient des concentrations inhibitrices à 50 % de plus de 0,036 $\mu\text{g./ml.}$, contre 46 % pour les souches de 1957. Les valeurs correspondantes pour 102 souches "à diagnostiquer" étaient 20 % et 17 % respectivement.

L'importance statistique de la différence entre les souches "à déterminer" et "à diagnostiquer" fut donc confirmée; en outre, on observa une tendance à une proportion plus élevée de souches moins sensibles (Figures 3 et 4).

Streptomycine: Les souches qui furent testées pour la pénicilline le furent également pour la streptomycine. Quand on employa la méthode de la tablette, le pourcentage des souches résistantes augmenta subitement jusqu'à 11 % en janvier et février 1960; avec la méthode de la dilution sur plaque, 26 % des 265 souches "à déterminer" étaient résistantes, tandis que seulement 10 % des 102 souches "à diagnostiquer" étaient résistantes (Figures 3 et 4). Pour obtenir l'inhibition complète des souches résistantes, il fallut plus de 2.000 $\mu\text{g./ml.}$

Tétracycline: 208 souches "à déterminer" et 102 souches "à diagnostiquer" furent testées par la méthode de la dilution sur plaque. Les résultats pour les deux groupes furent à peu près les mêmes que ceux observés pour les souches isolées en 1957. Cependant, on a remarqué récemment une tendance à l'augmentation de la concentration inhibitrice à 50 % parmi les 351 souches "à déterminer" reçues d'octobre à décembre 1960. Pour 4 % des 208

souches "à déterminer" isolées avant cette période, il fallut tout de suite $\geq 1,05$ $\mu\text{g./ml.}$ pour obtenir une inhibition de 50%, tandis que pour 10% des 351 souches isolées récemment, il fallut employer la même dose. (Figures 8 et 9.)

Sulfathiazole: L'auteur a passé en revue les recherches précédentes (surtout scandinaves); elle les a présentées, ainsi que ses derniers résultats, dans le Tableau et la Figure 10. En 1954, le niveau de sensibilité était à peu près le même qu'avant l'introduction des sulfamides.

Quand la méthode de la dilution sur plaque fut employée, les variations des 265 souches "à déterminer" s'étendaient sur un champ de près de $\times 200$. 3% étaient résistantes et nécessitèrent ≥ 48 $\mu\text{g./ml.}$ pour une inhibition de 50%; 31% étaient "moins sensibles" et nécessitèrent entre 8,5 et 48 $\mu\text{g./ml.}$ pour une inhibition de 50%. Les

valeurs correspondantes pour les 102 souches "à diagnostiquer" étaient 1% et 38% respectivement (Figures 8 et 9).

Corrélations: On constata une corrélation positive et importante du point de vue statistique entre les concentrations inhibitrices des 4 antibiotiques. En outre, il est utile de mentionner que pour 68 souches "à déterminer", plus de 2.000 $\mu\text{g.}$ de streptomycine par ml. furent nécessaires à l'obtention de l'inhibition complète; pour les mêmes souches, il fallut administrer au moins 0,15 $\mu\text{g.}$ de pénicilline par ml. pour obtenir l'inhibition complète.

On discute l'importance, du point de vue thérapeutique, de l'incidence plus élevée de souches moins sensibles et résistantes. On souligne que le médecin, le pharmacologue et le bactériologiste devraient collaborer afin d'utiliser le mieux possible les avantages des antibiotiques modernes.